Antioxidant enzyme overexpression protects cells with a mutation in succinate dehydrogenase subunit C (SDHC) from low dose irradiation radiation.

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Mitochondria are responsible for generation of substantial amounts of superoxide caused by one electron reduction of oxygen from the mitochondrial electron transport chains (METCs). Chronic metabolic oxidative stress can induce genomic instability and it has been suggested that METCs may participate in this process. Results from our lab have shown that Chinese hamster fibroblasts carrying a mutation in the METC protein, SDHC (B9 cells), produce excess superoxide and hydrogen peroxide as well as becoming genomically unstable, relative to parental B1 cells expressing wild-type SDHC. Preliminary results from our lab have also shown that B9 cells carrying mutations in SDHC demonstrate increased sensitivity to low dose (≤ 10 cGy) ionizing radiation (IR). *We hypothesized that overexpressing specific antioxidant proteins (superoxide dismutase and catalase) that detoxify superoxide and hydrogen peroxide would protect B9 cells from increased sensitivity to low dose IR.*

In the current study, mitochondrially targeted-catalase (MitCAT) protein, and MnSOD protein, were overexpressed in both B1 and B9 cell lines using adenoviral vectors. Wild type B1 cells treated with AdEmpty, AdMnSOD, AdMitCAT at 0 Gy and 0.1 Gy showed higher plating efficiency (PE) at 0.1 Gy than at 0 Gy (Fig. 1-A). This demonstrates that overexpressing antioxidant proteins and exposure of cell to low dose IR were nontoxic to the parental cells (Table 1 and 2; Fig. 1-A). In contrast, the B9 cells were significantly more sensitive to cell killing by 0.1 Gy of IR (Fig. 1-B). Furthermore, overexpression of catalase and/or MnSOD activity completely blocked the cytotoxicity of 0.1 Gy in B9 cells (Fig. 1-B). Likewise, treatment of B9 cells with polyethylene glycol (PEG) conjugated superoxide dismutase or catalase also blocked the cytotoxicity of 0.1 Gy radiation (Fig.2). These results show that overexpressing antioxidant enzymes that scavenge superoxide and hydrogen peroxide protect cells carrying the SDHC mutation from cell killing induced by low dose IR. These results support the hypothesis that mitochondrial production of superoxide and hydrogen peroxide could significantly contribute to the biological effects of low dose IR.

Table 1: Catalase activity (mkunits/mg) of B1 and B9 cells infected with adenoviral vectors.

Cell line	Cells alone	AdEmpty (50 MOI)	AdMitCatalase (50 MOI)	AdMitCAT+AdMnSOD (25 MOI ea.)
B1	4	4	17	10
B9	4	3	13	6

Table 2: MnSOD acitivity (units/mg) of B1 and B9 cells infected with adenoviral vectors.

Cell line	Cells alone	AdEmpty (50 MOI)	AdMnSOD (50 MOI)	AdMitCAT+AdMitMnSOD (25 MOI ea.)
B1	6	9	71	50
B9	13	12	29	21

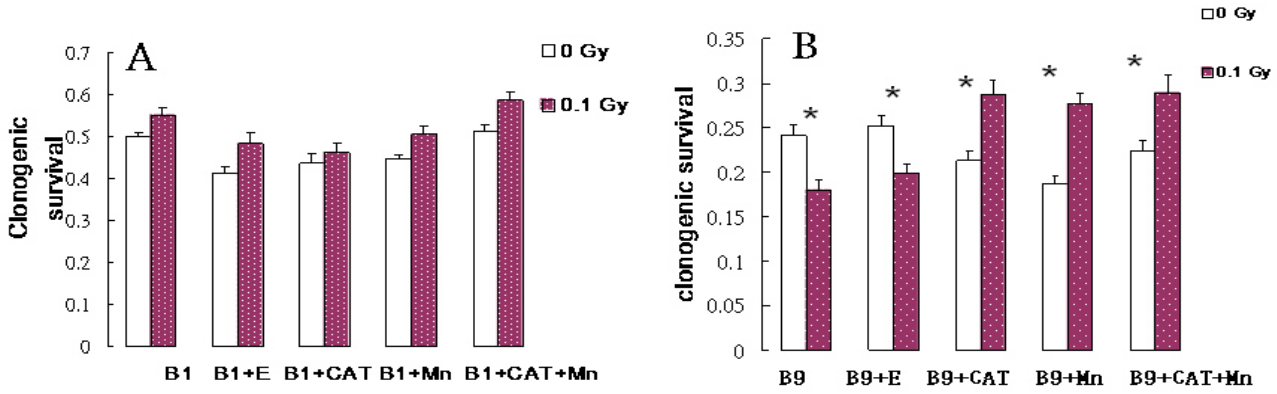

Fig 1: The plating efficiency of B9 cells irradiated with 0.1 Gy was increased by antioxidant enzymes overexpression. Figure 1 shows B1 cells (**Panel A**) or B9 cells (**Panel B**) exposed to low dose radiation with or without overexpression of catalase and/or superoxide dismutase, E= AdEmpty vector, CAT= AdMitoCAT, Mn=AdMnSOD. For the B9 cells (**Panel B**), there is a significant difference between unirradiated and irradiated cells in each treatment group. $P < 0.001$ * .The error bars represent 1 ± SEM from 3 separate experiments.

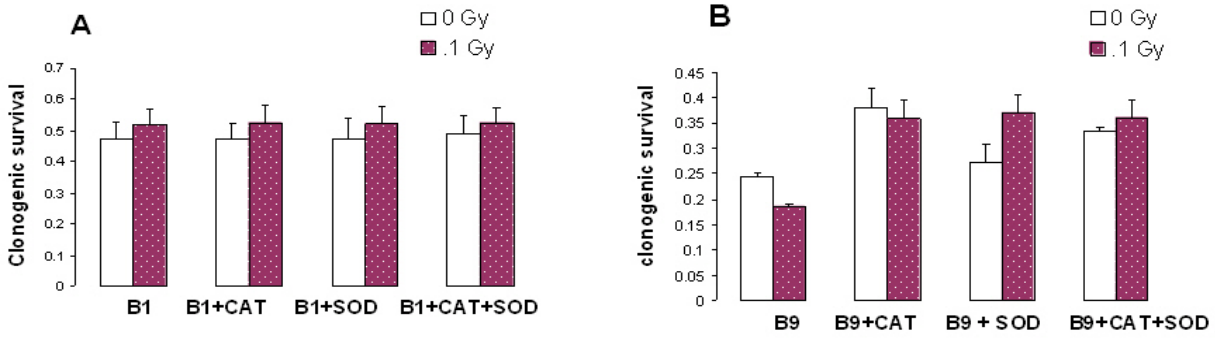

Fig.2: The plating efficiency of B1 (**Panel A**) and B9 (**Panel B**) cells at 0.1 and 0 Gy pretreated 4 hr with polyethylene glycol conjugated of superoxide dismutase and/or catalase. The error bars represent 1 ± SD from 2 separate experiments.